

TUTTLE (A. H.)

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THE

RELATION OF THE EXTERNAL MEATUS, TYMPANUM,
AND EUSTACHIAN TUBE

TO

THE FIRST VISCERAL CLEFT.

By ALBERT H. TUTTLE

WITH TWO PLATES.



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III.

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No. II. — THE RELATION OF THE EXTERNAL MEATUS, TYMPANUM, AND EUSTACHIAN TUBE TO THE FIRST VISCERAL CLEFT.*

BY ALBERT H. TUTTLE, BOSTON.

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THE recent revival of Von Baer's views† upon the development of the accessory parts of the ear in mammals again puts the question of their possible connection with the first visceral cleft in an unsettled condition. Such being the case, a reinvestigation of the problem seems desirable. It has been the aim of the author in the present paper to go over the ground in the case of the pig, and, with the aid of a more continuous series of embryos and improved methods of investigation, to ascertain in how far this recently revived theory is worthy of acceptance.

HISTORICAL SYNOPSIS.

In 1825 Rathke ('25, col. 747-749)‡ discovered, in the case of an embryo pig half an inch in length, that on either side of the neck immediately behind the head there were four gashes. These were successively smaller from before backwards, and all had a transverse or dorso-ventral direction. It was shown by dissection that they extended through the walls of the throat into the pharyngeal cavity. He accordingly called them "Schlundspalten" (throat clefts). At the same time

* Prepared in the Embryological Laboratory under Dr. E. L. Mark.

† Especially by Hunt and Urbantschitsch.

‡ At page 130 will be found a list of the papers cited, the names of authors being arranged alphabetically, the papers of each author chronologically. In the body of the paper the number immediately following the name of the cited author is an abbreviation for the date of the article referred to, and will enable the reader to turn at once to the full title of the paper.

he insisted upon their homology with the gill slits, especially of sharks, and therefore also designated them "Kiemenspalten" (branchial clefts). An embryonic horse two thirds of an inch long, which had lain some time in spirit, was taken for comparison. Although the external wall of the throat was smooth, the inner surface showed on either side four moderately deep furrows, having the same general form and position as the clefts in the case of the pig. Rathke thereupon came to the conclusion, that the embryos of mammals generally are provided with gills, and that the clefts are gradually closed by a fusion of their walls, which progresses from without inwards. This was, in substance, the important investigation which Von Baer afterwards alluded to as "Rathke's brilliant discovery of gill clefts."

Huschke ('26, col. 620) communicated to the assembly of German naturalists in September, 1825, his studies on the development of the frog and other amphibia. In the frog the gill apparatus becomes the middle ear, in that the inner opening of the first cleft becomes converted into the Eustachian tube. Huschke ventured to go farther, and to predict that in birds and mammals the first of the clefts recently discovered by Rathke becomes converted into the external meatus.

In the following year Huschke ('27, col. 401) extended his observations to the chick, where he found that between the two arches (mandibular and hyoid) there was a large hole which opened only a little farther back than the first gill cleft, and also at first led into the mouth cavity. It was no longer a gill opening, but external meatus, whereby it was in his opinion still better (than in frogs) established that this opening (i. e. as well as the Eustachian tube) possesses in the branchial fissure its first outline.

Rathke ('28, col. 80-85) soon after reported that he had not seen this hole, and he expresses a doubt as to its being the external meatus, even if it does exist.

Huschke subsequently ('28, col. 162) reaffirmed his interpretation of this opening in an article accompanied with illustrations. From the fourth to the fifth day a fine hair could be passed *without resistance* through this opening and the Eustachian tube into the throat and mouth, from which he inferred that the tympanic membrane was not yet formed. Von Baer ('28, p. 77), describing the chick of the fourth day of incubation, simply says that he observed a deep depression in the bottom of the pharynx pointing toward the ear, and that it was probably the beginning of the Eustachian tube. Early in the fifth day the first gill slit becomes unrecognizable (p. 83). The ear is indicated by a round elevated ridge, but the depression which it surrounds

remains very inconspicuous during the fifth day. Although the ear appears to have at this time an opening inward by means of the Eustachian tube, the external orifice is ordinarily formed on the following (sixth) day, so that it makes its appearance when the gill slits are closed. Still it was sometimes seen when one or the other of the clefts remained (p. 87). In the same year he (Von Baer, '28^a, p. 147) criticised Huschke's view in the following words: "The circumstance that the most anterior gill cleft is early reduced in length, and that its upper persists longer than its lower part, appears to have induced Huschke to regard it as the opening of the ear. So much is certain: the outer orifice of the ear can have nothing in common with the gill apparatus, since the ear belongs to the superior half of the animal (i. e. the Rückenplatte), and not to the inferior half (Bauchplatte), to which the gills belong." Von Baer admits, however, that the Eustachian tube is a prolongation of the superior half of the body into the inferior part.

In answer to this criticism of his theory of the transformation of the first cleft, Huschke ('32, pp. 40, 41) declared that Von Baer was in error in saying that the entire ear belongs to the superior half of the body; only the labyrinth is formed from it, the accessory parts being formed by the metamorphosis of the superior part of the first cleft. Huschke supported his position by renewed investigations, and by arguments based upon malformations observed in certain cyclopean monsters. The first pair of clefts, at first widely open, becomes, he says, progressively closed from the ventral median line backwards and upwards, and thus become widely separated, until at last only the outer or upper angle of each fissure is left as a hole, — the *meatus auditorius externus*, — which leads directly into the throat by means of the Eustachian tube without any tympanic enlargement.

Valentin ('35, p. 211) concludes from his own observations that it is not to be doubted that the Eustachian tube is the remnant of the inner end of the first visceral cleft, but is uncertain whether the tympanic cavity and the external meatus are "formed out of the whole external part of the cleft." His objection is based on the fact, that the first indication of the external opening of the ear does not lie in a line with the fused lips of the cleft, but "apparently above it in the substance of the posterior boundary of the first visceral arch."

Reichert's ('37, pp. 149-155) studies were made principally on the pig. He was the first to follow the successive changes of the hyo-mandibular cleft through a numerous series of embryos.* In many

* It was Reichert, also, who introduced the terms "Visceralbogen" and "Visceralspalten."

points concerning the external features of the cleft I have been able to confirm his conclusions. Soon after the confluence of the arches on the ventral side, says Reichert, there appear on the margins of the arches which bound the remnant of the first cleft elevations, the apices of which are directed toward each other; each of these elevations lies between two slight depressions. The cleft is thereby made to appear broader at two places (the ends), and narrow in the middle. In the further metamorphosis of the parts there are two points of importance: the changes of these elevations and depressions, and the actual and apparent migration of the external opening of the cleft. In stages where the second and third clefts are already closed, it can be ascertained by manipulation that the tissue which seals up the first cleft lies, as Rathke claimed, nearer the external than the internal opening of the cleft. Of the two broadened ends of the cleft, the lower (ventral) becomes the deeper and more important.

As seen from the side, the arches present, beside the two elevations mentioned, other smaller elevations and depressions, which together with the former surround the visceral opening like two walls. While the lower division of the cleft is continually becoming more evidently metamorphosed into the external meatus, the posterior wall, which is really an outgrowth of the second visceral process, gradually becomes higher, and while its main elevation forms, as it were, the root or stem of the external ear, the rest of it becomes the concha. The anterior wall does not become prominent.

As regards the second point, the remnant of the cleft at first lies between the upper (dorsal) portions of the first and second visceral processes, and it continues to occupy this position in relation to the forming parts; the latter, however, are shifted backwards by the formation of the lower jaw and the tongue, and in consequence of this the plane of the fissure, which was at first nearly perpendicular to the long axis of the embryo, becomes gradually more oblique. The apparent dorsal migration of the external opening, on the other hand, is due to the formation of the facial parts, especially to a gradual broadening of the ventral portion of the embryo in the ear region.

The inner portion of the cleft is elongated into a canal by the growth of the surrounding tissue. The canal is somewhat narrowed by the encroachment of the labyrinth near the place where the fusion of the walls of the cleft first took place; the portion lying outside the constriction becomes the tympanum, the rest becomes the Eustachian tube. The latter does not diminish in size, as Valentin claimed, nor is its axis changed in direction, but remains from the beginning directed, from outwards and forwards, inwards and backwards.

While Günther ('42, pp. 32, 56) agrees with Reichert as to the division of the external portion of the cleft into two parts, he disagrees entirely as to the fate of these parts. The edges of the first cleft fuse at first in the middle, so that instead of one there are two openings, the lower (ventral) of which suffers no metamorphosis and soon disappears entirely. The upper orifice is converted, the outer parts into the external parts of the ear, the inner into the tympanum and Eustachian tube.

In his text-book Bischoff ('42, p. 410) follows in the main the conclusions reached by Reichert, but, contrary to that author as well as to Rathke, he places the partition which separates the inner from the outer part of the cleft midway between the two ends, "in the middle of the thickness of the two arches."

According to Kölliker ('61, pp. 321, 322), the first cleft is closed in the human embryo in the fifth week; not, however, in its totality, like the other clefts, but so that on both sides of the place of union — which lies near the external orifice — the outer and inner ends remain open, and are respectively nothing else than the beginning of the external meatus and of the Eustachian tube and tympanum. The region of the union represents the primitive tympanic membrane. Subsequently the inner part of the cleft increases in length, and gradually becomes wider at its hinder or outer end.

In his lectures Rathke ('61, p. 117) taught that the first cleft in frogs and many toads, as well as in turtles and saurians, is only closed near its outer end, and from the substance effecting the closure is formed the tympanic membrane; in the case of birds and mammals, however, that it is closed at about the middle of its depth, and that the outer half of the cleft becomes the external meatus, while the inner becomes the Eustachian tube and tympanum.

In 1876 Hunt presented to the International Otological Congress a paper embracing "a new account of the development of the meatus externus, drum, and Eustachian tube." The whole paper was reprinted in the following year (Hunt, '77), and the substance of so much of it as relates to the meatus externus, etc. was also embodied in a paper published by the author in the same year in the *American Journal of the Medical Sciences* (Hunt, '77^a).

In these papers Hunt ('77^a, p. 1) says: "At the same time Von Baer stated an opinion as to the development of the meatus and Eustachian tube that was opposed by Huschke; here also the latter was declared victor, but I believe incorrectly." Hunt maintains that the external meatus is derived, at least in part, from a furrow formed at

the boundary of the swollen root of the first branchial arch and the neighboring tissue. "The Eustachian tube," he says ('77, p. 9), "is an involution of the mucous membrane lining the pharynx; it is not in any way the remains of a branchial fissure, but lies in the tissue in which the base of the skull is forming; it grows in length as the branchial fissure closes." He states that in cross sections of the head, which incline anteriorly as they pass downward so as to cut the first arch obliquely, the Eustachian tube can be seen as a slight depression in the roof of the pharynx. In an embryo nine sixteenths of an inch long it becomes deeper, pointing upwards and outwards. In an embryo eleven sixteenths of an inch in length the Eustachian tube overlaps the inner end of the meatus. The tissue separating the meatus and Eustachian tube becomes transformed into the tympanic membrane.

About the same time the development of the accessory parts of the ear was made the object of special studies by Moldenhauer and Urbantschitsch. Moldenhauer ('76) published a preliminary account of his results in the latter part of 1876; his conclusions will be given a little further on.

The studies of Urbantschitsch ('77) were made upon the rabbit and the chick. Supporting his statements (p. 4) by well-known conditions found in longitudinal sections of batrachians, he claims that the ridge-like thickenings of epithelium which mark the boundary between the fore-gut and the oral invagination (i. e. between entoderm and ectoderm) correspond exactly in position with the subsequently developed larynx. The oro-naso-palatal sinus in birds and mammals, which is at first much less pronounced than that of the lower vertebrates, is only definitely formed when the front part of the brain has come to form an angle with the axis of the rest of the body. It is then that the branchial arches begin to form by their downgrowth the lateral boundaries of this sinus, which is converted into the definite oro-naso-palatal cavity by the ventral union of the arches. The branchial fissures, as well as the inner wall of the arches and all other parts of this cavity, are therefore lined with *ectoderm*. The usual statement that the fissures are closed by the concrescence of the separate arches is not accurate; the closure of the clefts is effected by a crescent-shaped outgrowth of the substance of the provertebra which lies opposite the dorsal end of the cleft (p. 6). This outgrowth covers, or fills up, a portion of the dorsal part of the cleft, and a similar growth from the ventral side diminishes its extent in the opposite direction. Thus the fissure is reduced to a small circumscribed opening, — a temporary communication between the oral cavity and the outside, — which, just before its complete disappearance,

is seen to have a more ventral position (p. 7). The evidence that the fissures are not closed by a direct fusion of the facing walls of the arches Urbantschitsch finds in the fact that the *width* of the cleft does not suffer any perceptible change in the different stages, whereas a reduction of its *length* is directly visible at a comparatively early stage. "Furthermore," he says, "one can easily find in successive cross sections, either that the space between two arches discloses only the apposed layers of the ectoderm, or that between these two layers there are products of the mesoderm. Where there is no union the section passes through the region of the branchial fissure; where the two apposed layers of the ectoderm are encountered, there the cross section passes through the most peripheral part of the crescentic outgrowth; and where the provertebral mass is found between the two layers of ectoderm, there we have the already completely closed branchial fissure."

While the fissures are thus closed by the advancing mesodermic encroachments, the oral sinus undergoes a differentiation into a central and lateral portions; the latter become enlarged, and communicate with the former only by narrow fissures; the lateral enlargement becomes the middle ear, and the fissure the Eustachian tube, so that in its origin the middle ear is in no way derived from the first branchial cleft, but is to be sought in the two lateral sinuses of the oro-nasopharyngeal cavity. The tympanic membrane as seen from the outside is at first not distinguishable, inasmuch as it lies in a plane with the external surface of the rest of the embryo; from the beginning it consists of three layers,—the external layer of ectoderm, the mesoderm, and the inner or oral layer or ectoderm (p. 13).

Only occasionally is its position indicated externally by a slight depression. This depression is the beginning of the external meatus, the entrance to which is separated from the first visceral cleft by a narrow bridge of tissue. Although this at first appears to lend support to the theory of its derivation from the first cleft, it is nevertheless not so derived. The orifice is the opening to a canal (meatus) which is entirely distinct from the cleft, and forms with it an angle the opening of which is directed inward and forward. This canal is formed by the elevation of a ridge of tissue around this depression, rather than by any ingrowth of the latter.

Urbantschitsch ('78, p. 132) in a subsequent paper reaffirms his conclusions, and claims that the external meatus is formed in the embryos of mice in a like manner.

By far the most careful and systematic description of the development of the middle and external ear is that given by Moldenhauer ('77)

for the chick. His account embraces a description of the changes as observed both from the outside and from the inner side of the branchial region, and is made as definite as possible through the aid of what is really indispensable, — series of sections both transverse and frontal.

In the chick of the fourth day the first cleft is already closed except in its posterior* third. The position of the fissure is, however, still recognizable by the presence of a furrow which is least conspicuous in the region of the middle third of the fissure, which latter, moreover, is the place in which fusion first occurs and which marks the position of the future tympanic membrane. The total length of the branchial furrow is .53 mm., of which the posterior open part occupies .17 mm.

By the sixth day the furrow has increased to .83 mm., but the posterior open portion remains unchanged in length, nor is it really open at this time, since deep down a welding has closed the passage. Two pairs of elevations — *colliculi branchiales externi* — arise from the edges of the first and second arches, and surround all of the furrow except a small anterior portion. Of the superior pair the posterior elevation corresponds in position to the root of the first arch, the anterior to the posterior end of the mandibular process. These elevations are so placed that the furrow is broken into a zigzag, the most important part of which, since it corresponds to the region of first closure, is a valley, the sides of which are bounded by the upper anterior and the lower posterior elevations, and the ends of which abut against the two remaining colliculi.

On the seventh and following days the colliculi become more prominent and the corresponding valley deeper; the lower posterior elevation soon begins, however, to become flattened, while the three remaining colliculi become confluent, and thus form a sharp lip, which slightly arches over the depression from the upper and anterior sides, and thus converts the latter into an oblique passage, the entrance to which is on the side of the flattened and finally obsolete colliculus.

The *inner* surface of the first arch in the chick of the fourth day is raised into an elongated elevation, — *colliculus palato-pharyngeus*, — which, beginning near the origin of the maxillary process, extends downward across the root of the first arch perpendicularly toward the first cleft. It gradually increases in prominence from above downward, and ends with a rounded margin at the lower edge of the first

* Moldenhauer's method of designating directions is borrowed from anthropotomy, so that his "anterior" refers to the *ventral* face of the embryo, and his "posterior" to the *dorsum*. For the same reason the cephalic end is called "upper" or "superior"; the caudal, "lower" or "inferior."

arch. This elevation is marked off from the rest of the arch by two grooves, both of which run into the first cleft at their lower ends; but in the upper part of their course they differ. The posterior one — *sulcus tubo-tympanicus* — gradually becomes shallower till, at its upper end, it is no longer distinguishable; the anterior, — *sulcus lingualis*, — however, bends forward at its upper end, and opens to the outside at the angle between the maxillary and mandibular processes. The latter subsequently bounds the outer margin of the tongue; the former is the beginning of the Eustachian tube and the tympanic cavity.

At its upper or basal portion the margins of the colliculus are thickened into crura; the posterior — *crus superius* — retains its perpendicular direction; the anterior — *crus inferius* — bends forward nearly parallel with the maxillary process, and is the first trace of the palatine arch. The edges of the superior crura of the opposite sides of the embryo subsequently fuse, thus forming the common sinus, characteristic of birds, into which the Eustachian tubes directly open.

Cross sections of a two-day embryo chick show that none of the clefts are formed, although the entoderm is in contact with the ectoderm in the region of the first cleft, preparatory to the formation of an opening. Similar sections of 3–4-day chicks — in which all four clefts are open — exhibit the *sulei* as narrow curved hornlike fissures. The dorsal horn, — *sulcus tubo-tympanicus*, — faintly indicated in more anterior sections, becomes deeper in the more posterior, and finally opens out through the first cleft. Its extent is shown by the position of the sections of the branchial artery; owing to its arched course the latter is twice cut in cross sections; the sulcus begins near the inner section of this vessel, where its presence causes the dorsal wall of the pharynx to protrude, and it extends outward not farther than the outer section of this artery.

On the sixth day the clefts are all closed except a small posterior portion of the first. The *sulcus tubo-tympanicus* has a bend which gives its outer end a dorsal direction. In a section through the branchial furrow, which cuts through the territory of the mandibular arch rather than the hyoid, there is in a line with the sulcus a slight depression of the ectoderm; immediately in front of this there is a prominent elevation (a section of the *colliculus posterior superior*), while in front of this elevation there is a second more deeply depressed region, — the oblique valley seen in surface views, — which terminates abruptly in front at a second elevation, the section of the *anterior superior colliculus*. This deeply depressed region lies opposite the bend in the sulcus, and corresponds to the thinnest part of the wall; it marks the place of the tympanic membrane.

In corresponding sections through embryos of the ninth day the posterior colliculus, as well as the depression behind it, no longer exists; the depressed region grows gradually deeper from behind (dorsal) forward, where it is terminated by a steep wall, — the anterior colliculus. An enlargement of the blind end of the sulcus opposite this depression is the incipient tympanum.

In an embryo of the twelfth day the external depression — the *meatus externus* — has become deeper; the deeper portion of its posterior wall is differentiated into a floor, the tympanic membrane, with which the anterior wall makes a sharp angle. The tympanic membrane is only half as thick as in the nine-day stage. Since it is not the dorsal depression, lying in line of the prolongation of the sulcus, which becomes the external meatus, but rather the more ventral depression, Moldenhauer argues that the tympanic membrane has no relation whatever to the blind end of the sulcus, its mucous surface corresponding to a definite territory of the *ventral* region of the wall of the front intestine. Similar conditions are maintained for the embryo of the deer.

From the study of *frontal* sections the author concludes that the tympanic cavity lies on the inner side of the first *arch*, since in the chick of the seventh day the *lower* (posterior) wall of the primitive tympanum lies nearly in continuation with the layer of epithelium which indicates the position of the line along which the arches fused. The tympanic membrane is formed at the expense of the substance of the mandibular arch, not that of the hyoid. This conclusion is based principally on the fact that the lower (posterior) margin of the first arch *gradually* becomes thinner in the tympanic region, and that the upper edge of the hyoid arch rises *abruptly* from the region of minimum thickness.

Kölliker ('79, pp. 746-755) still holds that the tympanum and tuba are undoubtedly developed from the median part of the posterior (dorsal) section of the first branchial fissure, which, however, is not directly and without further change metamorphosed into these parts, but which grows out into a hollow process directed outward, upwards (forward), and backwards (dorsal). At the same time the external meatus, which at first is shallow, forms also a hollow process which takes exactly the opposite direction; the meatus therefore does not become deeper simply from the upgrowth of the tissue surrounding its external opening. Kölliker finds in the rabbit nothing to favor Moldenhauer's view that the mouth of the tuba and that of the first cleft do not correspond, neither is he willing to commit himself to the opinion which makes the

tympanic membrane arise exclusively from the substance of the mandibular arch.*

OBSERVATIONS AND CONCLUSIONS.

Does the external passage of the ear arise by a transformation of the first, or hyomandibular cleft? This question may be fully answered by observing the external changes in a series of embryos. It is to these external changes that I shall first direct attention. There are three possible *a priori* answers to the question: first, the external passage may be developed exclusively from the remnant of the cleft, simply by an outgrowth of its walls; secondly, it may arise quite independently of the cleft, by means of a new invagination and subsequent changes; or, finally, it may owe its origin in part to a transformation of the cleft and in part to other and subsidiary changes of parts not belonging to the walls of the cleft proper.

The smallest embryo I have studied in this connection is 6 mm. long;† its form is much more simple than that of any of the later stages. The visceral arches are straight, finger-like processes, without elevations or depressions. The clefts are also straight and deep, with perfectly smooth walls; in the profile view of the embryo they extend dorsally for nearly half the breadth of the neck (Fig. 1). In this embryo only the mandibular and hyoid arches have become well defined. The otic vesicle is still traceable by an external depression due to a sinking in of its thin outer wall during the process of hardening.

In the growth of an embryo from the 6 mm. to the 8 mm. stage (Fig. 2), a considerable alteration takes place in the appearance of the head and neck. The different regions of the brain have become well marked externally; the third visceral arch is sharply defined; the mandibular and hyoid arches are swollen at their roots. The furrow described by Hunt as lying above the root of the mandible has become perceptible (Fig. 2, *d H*). The mandibular and hyoid arches have exchanged their finger-like appearance for somewhat club-shaped forms. The appearance of the first cleft has also become altered; formerly

* Unless I have myself misunderstood the figures and meaning of Moldenhauer, Kölliker is in error when he makes the latter responsible for the view that the external meatus is derived from the *most posterior* (dorsal) part of the cleft. I understand Moldenhauer to say that it is not the dorsal depression corresponding to the last closed part of the cleft, but a more ventral region of the cleft, which marks the position of the future meatus and its floor,—the tympanic membrane.

† Measurements were made in a straight line from the elevation of the mid-brain to the most distant point of the embryo as it lay in its natural position.

straight, it is now bent near the middle by an elevation formed on the anterior margin of the hyoid arch (Fig. 2, *p e*), which projects over the cleft. This elevation overlaps a depressed portion of the mandibular arch, and plays an important part in the development of the auricle of the ear.

In an embryo 9 mm. long (Fig. 3) the cleft has become more bent by the forward growth of the elevation of the hyoid arch, and the deepening of the corresponding depression of the mandibular arch. The furrow of Hunt has become limited to a small triangular depression, the ventrally directed apex — the part corresponding to the dorsal end of the cleft — being the deepest. At this stage the depression is quite conspicuous, since the roots of the hyoid and mandibular arches, which form its ventral walls, are much swollen. The elevation on the hyoid (Fig. 3, *p e*) has become more prominent, and has thus affected the shape of the first arch, the mandibular branch of which, as seen in profile, has taken an almost rectangular outline.

With embryos 10 mm. long (Fig. 4) the cleft is still more bent, and is sharply limited by the hyoid wall. Its anterior margin, however, is not so sharply marked, since the mandibular wall gradually slopes into the cleft. At either side of the hyoid elevation the cleft is deepest, becoming more shallow as one approaches its extremities. The elevation on the hyoid has increased considerably in size, and at either side of it is formed a secondary elevation (*s d, s v*). Each of these, although very slight as yet, is clearly distinguishable, and is separated from the primary elevation by a shallow depression (*d*). On the mandibular arch opposite these elevations there are others (*m d e, m v e*) which form the angles of that arch: they give a definite limitation to the cleft, and at the same time clearly define the rudimentary jaw. The depression of Hunt has become less pronounced because of the outward growth of the tissue forming its floor, owing to the more complete fusion of the roots of the mandibular and hyoid arches.

In embryos 11 mm. long (Fig. 5) the primary elevation of the hyoid has assumed a conical shape; it is thickened and swollen at the base, but thinned out at the apex, and is very sharply limited. The secondary elevations have become distinctly separated from the primary by the deepening of the depressions between them and the latter, and have thus become much more prominent. At either side of the primary elevation of the hyoid the cleft has the appearance of a deep hole, which is sharply bounded by the almost perpendicular walls of the hyoid and mandibular arches. The elevation at the root of the mandibular arch is still prominent, whereas the depression of Hunt is almost obliterated, at least in embryos prepared by the picro-sulphuric acid process.

A series of embryos from 11 mm. to 17 mm. long shows the gradual disappearance of the elevation at the root of the mandible and of the depression of Hunt. The primary elevation of the hyoid arch (Fig. 6) becomes more and more developed, and its growth is attended with apical attenuation. The secondary elevations become less conspicuous, and show a tendency to coalesce with the primary. The superior (dorsal) one, however, is still distinct. The mandibular wall continues to slope rather gradually into the cleft. The latter is now confined to the side of the head, and the surrounding parts show some resemblance to the permanent external ear; the apex of the primary elevation of the hyoid arch corresponds to the tip of the auricle.

In the case of an embryo 19 mm. long (Fig. 7), the walls of the *meatus* (for such we may now call it) are steep and sharply limited. The inferior (ventral) secondary elevation of the hyoid has disappeared, whereas the superior one still exists.

When the embryos have attained the length of 25 mm. (Fig. 8) the auricle has a triangular shape, thus approximating its ultimate form. The superior secondary elevation has by this time disappeared, and the whole aspect now closely resembles that of the ear of the animal at birth.

From this description it will be seen that the development of the auricle is the most salient feature of the external changes in the walls of the cleft, and is distinguishable at a much earlier epoch than has hitherto been claimed. It is to be seen first in an embryo 8 mm. long as a slight elevation of the anterior margin of the hyoid arch (Fig. 2, *p e*). This elevation gradually increases in prominence, but always preserves its primitive relation to the cleft, since it continues to point directly across the centre of the latter. In an embryo 25 mm. long the centre of the meatus is directly beneath the apex of this auricular bud (Fig. 8). Inasmuch as the centre of the cleft of an 8 mm. embryo occupies the same position in relation to the incipient auricle, it is fair to infer that the external meatus occupies the region of the centre of the cleft.

The long diameter of the slit does not increase to any great extent until the embryo is 30 mm. or 40 mm. long, although it increases rapidly in width after the embryo attains a length of 17 mm. From this it would seem that the depression described by Hunt (which lies at the dorsal extremity of the cleft, and owes its existence to the less rapid growth of the tissue at that point), instead of deepening, becomes obliterated by a thickening of the tissue which forms its floor. This thickening constitutes the wall bounding the dorsal extremity of the cleft. In other words, the inclined floor of this depression forms the

dorsal wall of the cleft, just as in many young stages the mandibular arch forms the sloping anterior wall of the cleft.

After bisecting an embryo 8 mm. long, and laying open the pharynx, the cavity of the first cleft may be viewed from the inside. It will be found to be a deep blind pocket extending dorsally and outwards, the opening to which is long and narrow, although somewhat wider near the centre. The inner openings of the other clefts also are still very conspicuous.

In an embryo 11 mm. in length the opening of the first cleft has become more diamond-shaped, and is sharply defined by a ridge which forms its dorsal and posterior margins. The remaining clefts, having become entirely closed, are now only recognizable as shallow furrows.

When the embryo has grown to 16 mm. in length these furrows have become almost obliterated. The ridge which forms the posterior and dorsal boundary of the first cleft is more prominent. By carefully reflecting it one may observe a bridge of tissue, the primitive tympanic membrane, through which a certain amount of light penetrates. A hair inserted from the outside through the middle of the floor of the external portion of the cleft will pass through this membrane at a point near its centre. From this it is evident that the external depression must lie directly opposite the internal part of the cleft, and that therefore the former is *not* a secondary depression at one side (dorsal) of the external part of the cleft, as claimed by Urbantschitsch.

An embryo 18 mm. long shows that the ridge still exists, and that it serves to define the pharyngeal opening of the first cleft; there remains, however, no trace of the other clefts.

In order to study the shape of the internal part of the first cleft, as well as the changes which it undergoes and its relation to the external portion of the cleft, it is necessary to employ a series of frontal and cross sections through this region at various stages of development. For this purpose embryos were killed in picro-sulphuric acid, and hardened in alcohol. They were stained *in toto* in Grenacher's alcohol-borax carmine. Several other methods of hardening and staining which I employed gave much less satisfactory results.

Sagittal sections through the head of an embryo 6 mm. long show that the walls which bound the first cleft in front and behind have already come in contact along a line running nearly parallel with the surface of the neck, but much nearer the superficial than the deep, or

pharyngeal, end of the fissure. This line of contact is coextensive with the cleft, so that the latter is closed throughout its whole length (Figs. 9, 10, 11),* although a complete fusion of its walls can hardly be said to have taken place at this time. The second cleft has not yet broken through the pharyngeal walls, so that for a short period subsequent to the formation of the first cleft there is no direct communication through the walls of the neck between the pharyngeal cavity and the outside world. The pharyngeal portion of the walls of the first cleft at this stage is composed of two layers of cells, a superficial layer of flattened epithelium, and a deep (or mucous) layer consisting of wedge-shaped or somewhat modified columnar cells. On the posterior or hyoid wall the deep layer is several cells in thickness, but on the anterior or mandibular wall this layer is usually only two, and at most not more than three, cells deep.

In cross sections through the centre of the cleft of an embryo 8 mm. long (Fig. 12), the ventral half of the external division of the cleft is separated from the internal portion by a thick partition consisting of three layers: the ectoderm lining the external portion of the cleft, the entoderm lining the internal portion of the cleft, and the mesoderm intercalated between them. The deepest portion of the external moiety of the cleft, however, which lies at its dorsal extremity and which coincides with the ventral angle of the depression of Hunt, is separated from the inner portion of the cleft by only a few cells which have been proliferated from the hyoid wall (Fig. 13). I believe this affords considerable evidence that the cleft is gradually closed from its ventral toward its dorsal edge by an upgrowth of tissue which results from a very complete fusion of the hyoid and mandibular arches.

The regions of the external portion of the cleft which play the most important part in the formation of the external meatus are indicated in the sections (Fig. 12) by *a* and *b*. They are a dorsal depression (*a*), which lies at the extremity of the cleft, and a ventral depression (*b*), which lies anterior to the primary elevation of the hyoid. From the same sections (Fig. 12) the inner portion of the cleft (*c*) is to be seen directed obliquely from the pharynx dorsally and outward. Since the inner portion of the cleft and the pharynx meet at an angle, the latter affords a well-marked indication of the extent of each. These characters are important in determining the cleft, and orienting its position in the successive stages of its metamorphosis.

Cross sections through the cleft of an embryo 12 mm. long (Fig. 14) show that the dorsal depression (*a*) has become deeper and more nar-

* Consult Explanation of Figures.

rowly limited, on account of the thickening of the walls of the head without any corresponding increase in the thickness of the floor of the depression. For the same reason the ventral depression has become deeper. This series of sections shows that an ingrowth of mesoderm has occurred even in the thinnest region of the partition, which so thickens it as to separate more than in the previous stage the inner from the outer portion of the cleft. The ingrowth is greater between the ventral depression and the inner portion of the cleft than it is between the dorsal depression and the latter. It reduces the inner remnant of the cleft to a narrow cavity, which, from its shape and position, we may call the inner edge of the original cleft; this persists as the tympanum and Eustachian tube, but it communicates with the pharynx proper only at its ventral end. That communication becomes the true Eustachian tube. At this stage it points directly from the pharynx to the dorsal depression (*a*), and the partition which separates it from the same is still very thin (Fig. 15).

By the time the fœtus has reached the length of 16 mm. the dorsal depression (Fig. 16) has become much shallower, since the bridge which separated it from the inner remnant of the cleft has become very thick. The ventral depression, on the other hand, has increased in depth. By these changes the floor of the external part of the cleft is placed in a line parallel to the internal portion of the cleft. The primitive tympanum and Eustachian tube continue to point toward the dorsal depression.

This is the stage that has misled many prominent investigators. The ventral depression, which has now become very pronounced, has been mistaken for the dorsal one, which at this time is quite inconspicuous on account of the upward growth of the tissue that forms its floor.

The length of the inner portion of the cleft, as Reichert first pointed out, has not suffered any perceptible increase; but, with a confusion of these external depressions, one might think there had been a dorsally and posteriorly directed evagination on the part of the blind end of the inner portion of the cleft. The dorsal part of this primitive tympanum is, however, somewhat enlarged.

The whole region of the external depressions is somewhat deeper in an 18 mm. embryo, and the ventral depression especially has become deep and narrow (Fig. 17). No other important changes are noticeable, except an increase in the obliquity of the plane of the partition, principally due to the deepening of the ventral depression.

When the embryo has reached the length of 25 mm. the ventral depression is much deeper (Fig. 18, *b*). It can now be called the exter-

nal meatus. Whereas its termination maintains the same, or nearly the same, relation to the pharyngeal cavity as before, it has approached much nearer to the inner portion of the cleft, inasmuch as the thickening of the walls of the head in this region has slightly straightened the latter, and brought its long diameter into a position more nearly perpendicular to the surface of the head. At the same time there has been a reduction in the thickness of the partition (primitive tympanic membrane) which separates the inner from the outer remnant of the cleft. This partition subsequently becomes still thinner (Fig. 20), but according to K  lliker and others it retains a considerable thickness during the whole of embryonic life.

To recapitulate, the external meatus is formed by the outward growth of the tissue constituting the walls of the first visceral cleft. The closure of the cleft is effected from its ventral edge toward its dorsal extremity by the gradual upgrowth of tissue, which in turn results from the fact that the fusion of the contiguous arches progresses dorsally. The position of the dorsal extremity, which is the last to close, is for some time marked by an external depression (*a*), but this afterwards becomes more shallow, whereas the ventral portion, which soon after the closure of the dorsal end of the cleft is indicated by only a shallow depression, gradually increases in depth, and ultimately forms the deep portion of the external meatus.

The tympanic membrane originates from tissue in the region where the cleft is first closed; it is not uniform in thickness at first, and it lies inclined to the surface of the embryo; afterwards its thickness is reduced, and at the same time becomes more uniform. Simultaneously the plane of the tympanic membrane is shifted, from a position approximately parallel to the surface of the head, to one more nearly perpendicular to that surface. The inner remnant of the cleft increases slightly in length, but probably not much, until the embryo is 18 mm. long; this, moreover, is not due to an actual evagination, but appears to be accomplished by the growth of the tissue forming its walls. Its blind end enlarges to form the tympanum, while the remaining less altered part forms the Eustachian tube.

The first cleft is closed at a much earlier stage than observers have hitherto claimed. The hyoid and mandibular walls come in contact along a line parallel to the surface of the embryo and a little nearer the external than the internal opening of the cleft. From the contiguous walls are proliferated cells which form the bridge across the cleft. Simultaneous with this process occurs the formation of the incipient auricle, the changes in the deeper portion of which soon separate the

remnant of the cleft into two distinct parts, a dorsal shallower and a ventral deeper part. Both of these persist: the ventral part is transformed into the deep portion of the meatus, and together with the superior half it also forms the cavity of the external ear. Therefore, as claimed by Reichert, the ventral division of the cleft plays the more important part in the formation of the external ear. In my observations I have been unable to find any evidence in favor of Günther's view that the walls of the cleft fuse in such a manner as to leave for a short period *two* openings into the pharynx. On the contrary, the bridge of tissue that closes the last remnant of the opening is a simple accumulation of cells, which forms an uninterrupted membrane of approximately uniform thickness. This remains for some time very thin, and might easily be ruptured even by as delicate a probe as a hair; moreover, the cleft does not show a definite division into a dorsal and a ventral part until the embryo is about 10 mm. long; but at this time the cleft has long been closed, although the bridge is still very thin. It is from these considerations that I have been led to believe that the two openings claimed by Günther were artificially produced by a rupture of the tissue forming the bridge.

As to the conclusions reached by Urbantschitsch about the manner in which the visceral fissures close, it is to be observed, in the first place, that his cross sections (*op. cit.*, Figs. 1-3) do not afford the least direct evidence that the first cleft remains pervious until it is reduced to a mere hole, for the sections only pass through the orbital process and the lower-jaw branch of the first arch. It is unfortunate for his claim, that he has not reproduced the sections which show that "the same condition that is encountered between the orbital process and the lower-jaw branch is found between each two of the arches." In the second place, the single section shown in his Fig. 4 is not in itself alone sufficient to prove that the cleft is not already closed by a continuous bridge of tissue. He calls this a frontal section: the regions of the embryo touched by the section seem, however, to indicate that it is not strictly frontal, but, on the contrary, quite oblique to that plane, — perhaps even nearer a sagittal than a frontal plane. The section is to all appearances approximately parallel to a tangent to the side of the head and neck in the region of the visceral arches and clefts, so that it cannot be safely inferred from the condition of the fissures in the plane of this section that they present the same condition in every part of their depth. The figures accompanying the present paper (Figs. 9, 10, 11) show how unreliable the conclusions based upon any single section, like that shown in Fig. 9, would have been. While, then, I do not doubt that the restriction of the fissure is largely accomplished, as

Urbantschitsch claims and as Reichert long ago stated, by the dorsal and ventral ingrowth of tissue, it remains none the less true, for the pig at least, that this is only a part of the process, and that an early fusion of the arches makes the first cleft impassable long before the reduction in the length of the fissure is effected by these mesodermic growths. Moreover, although I admit the dorsal ingrowth to be subsidiary to an increase in the thickness of the partition that closes the cleft, it helps only after the bridge has been formed by cells derived from the hyoid arch.

The conditions presented in the chick, as shown by Moldenhauer's studies, and those in the pig, are in many points strikingly similar; but it is the *interpretation* given to the facts by Moldenhauer which is at variance with my own conclusions. The real question at issue is this: Is any part of the tubo-tympanic sulcus a result of the direct metamorphosis of the first cleft, or is it rather the result of a secondary outgrowth from pharyngeal cavity? Moldenhauer defends the latter assumption, whereas I am convinced of the validity of the former. To answer the question, it seems to me only necessary to determine the extent of the tubo-tympanic sulcus in two directions, — the dorsal and the posterior.* According to his own figure (Moldenhauer, '77, Taf. VIII. Fig. 23), with which my own on the pig (Fig. 16) agrees in all essential matters, the dorsal limitation (blind end) of the sulcus corresponds quite accurately with the dorsal extent of the fissure before its closure. The depression of the ectoderm dorsal to his colliculus posterior, through which the lines *ab* pass in his Fig. 23, lie, according to his own description (p. 130), in the prolongation of this sulcus; but the depression in question corresponds approximately to the dorsal limit of the now closed cleft. That this depression does not subsequently grow deeper and thus become the external meatus, as has been supposed by some of the earlier observers, but is finally obliterated, can be no argument against its being the remnant of the dorsal region of the primitive cleft; if it is such, then it is certain that the sulcus tubo-tympanicus does not have a greater *dorsal* extension than would be expected if it were simply the remnant of the inner half of the first cleft. There is, then, no motive for assuming a secondary outgrowth in a *dorsal* direction.

As regards the anterior (upward, Moldenhauer) extent, it certainly must be admitted to be at first greater than that of the fissure; its posterior end, however, communicates at an early stage with the outside by

* Posterior and superior respectively, according to Moldenhauer's terminology.

means of the first cleft; in other words, the posterior (lower, Mold.) region of this sulcus is identical with the inner end of the fissure, which in turn is continuous with the pharyngeal cavity. The question, then, simply becomes, Which portion of this sulcus is converted into the Eustachian tube and drum,—the anterior or posterior? When it is observed that the greatest lateral extent of the sulcus is encountered in the cross sections (Moldenhauer's Figs. 23, 25) which lie *in the plane of the branchial furrow*, I think there need be no hesitancy in admitting that it is the *posterior* part of this sulcus which persists, and is immediately concerned in the formation of the drum and tuba; and if so, there can be no valid objection to considering these spaces as resulting from the metamorphosis of the inner edge of the first visceral cleft.

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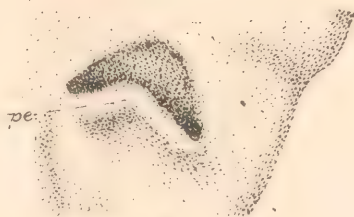
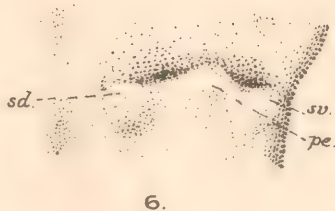
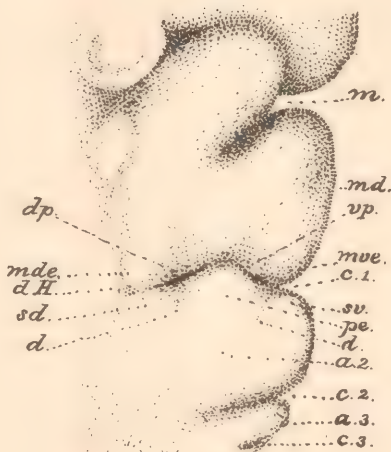
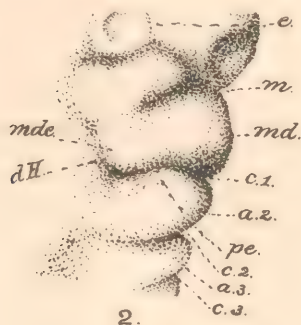
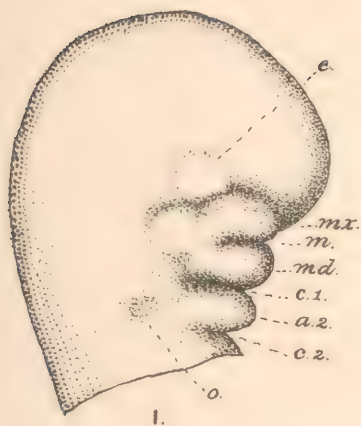
EXPLANATION OF LETTERS.

- a. Dorsal part of the first visceral cleft, from Hunt's depression to the point where it becomes continuous with the ventral depression of the cleft.
- a. 2. The second visceral or hyoid arch.
- a. 3. The third visceral arch.
- b. The ventral portion of the cleft to its union with the dorsal part.
- c. The inner portion of the first visceral cleft.
- c'. The inner portion of the second visceral cleft.
- c. 1. The first visceral or hyo-mandibular cleft.
- c. 2. The second visceral cleft.
- c. 3. The third.
- d. Depressions separating the primary from the secondary elevations.
- d'. Angle marking the limit between the cleft and pharynx.
- d H. Depression described by Hunt.
- d p. Dorsal depression of the first cleft.
- e. Optic vesicle.
- m. Mouth.
- m d. Mandibular arch.
- m d e. Dorsal mandibular elevation; the swollen root of the mandible.
- m e. Meatus externus. (In early stages the external portion of the first visceral cleft.)

- m e s.* Mesoderm.
m v e. Ventral mandibular elevation.
m x. Maxillary branch of mandibular arch.
o. Otic vesicle.
p e. Primary elevation on the hyoid arch.
s d. The dorsal secondary elevation of the hyoid arch.
s v. The ventral secondary elevation of the hyoid arch.
v p. Ventral depression of the first cleft.

EXPLANATION OF FIGURES.

- Fig. 1. Head and neck of an embryo pig 6 mm. in length.
 Fig. 2. Side view of an embryo 8 mm. long, in the region of the clefts.
 Fig. 3. Same view of an embryo 9 mm. long.
 Fig. 4. Same, of an embryo 10 mm. long.
 Fig. 5. Side view of the first cleft of an embryo 11 mm. long.
 Fig. 6. The same region from an embryo 17 mm. long.
 Fig. 7. The incipient auricle at a stage 19 mm. long.
 Fig. 8. The same at a 25 mm. stage.
 Figs. 9-11. Three successive sections tangential to the left side of the neck, cut nearly parallel to the sagittal plane, from an embryo 6 mm. long. The sections pass through the region of the first and second visceral clefts. The distance of the nearly parallel lines indicates the relative thickness of the ectoderm.
 Fig. 12. The outlines of a series of sections through the region of the cleft of an embryo 8 mm. long. (Since the complete series is quite numerous, only a certain number have been selected for reproduction. Their positions in the series are indicated by the numbers accompanying each outline. The sections are cut somewhat obliquely to the plane of the cleft: they incline from above downwards and backwards. The successive sections from the left to the right correspond to successive planes from before backwards.)
 Fig. 13. A cross section of an embryo 8 mm. long, which shows the bridge of epithelial cells that separates the inner from the outer portion of the cleft.
 Fig. 14. A series of cross sections through the region of the first cleft of a 12 mm. embryo.
 Fig. 15. Frontal section of the first cleft of a 12 mm. stage, showing the mesoderm which increases the thickness of the bridge.
 Fig. 16. A series of sections from an embryo 16 mm. long, cut parallel to the cleft.
 Fig. 17. A series of sections, also parallel to the cleft, from an 18 mm. embryo. They show a deepening of the ventral depression, and a change in the direction of the plane of the bridge which separates the inner from the outer portion of the cleft.
 Fig. 18. Transverse section through the region of the ear from an embryo 25 mm. long. The bridge has become thinner, and its long axis is now nearly perpendicular to the surface of the head.
 Fig. 19. A portion of the section shown in Fig. 20, more highly magnified.
 Fig. 20. Section from a more advanced embryo (30 mm.), made in the same direction as in Fig. 18, but a little more posterior.



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